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UTILITY PATENT APPLICATION TRANSMITTAL

Utility for new nonprovisional applications under 37 CFR 1.53(b)

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Inventors: Anders BERKENSTAM and Mats DAHLBERG			
Title: Novel Vitamin D Receptor Related Polypeptides, Nucleic Acid Sequence Encoding the Same and Uses Thereof			
Express Mail Label No.	EM382373490US		

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents

1. ☒ Utility Patent Application Transmittal [Total Pages: 2]
2. ☒ Specification, Claims and Abstract [Total Pages: 22]
3. ☒ Drawing(s) (35 USC 113) [Total Sheets: 15]
4. Oath or Declaration [Total Pages:]
 - a. ☐ Newly executed (original or copy)
 - b. ☐ Copy from a prior application (37 CFR 1.63(d)) (for continuation/divisional with Box 17 completed) [Note Box 5 below]
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Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).
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6. ☐ Microfiche Computer Program (Appendix)
7. ☐ Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary)
 - a. ☐ Computer readable copy
 - b. ☐ Paper copy (identical to computer copy)
 - c. ☐ Statement verifying identity of above copies

Accompanying Application Parts

8. ☐ Assignment Papers (cover sheet & document(s))
9. ☐ 37 CFR 3.73(b) Statement ☐ Power of Attorney (when there is an assignee)
10. ☐ English Translation Document (if applicable)
11. ☐ Information Disclosure Statement (IDS/PTO-1449)
 - ☐ Copies of Citations
12. ☐ Preliminary Amendment
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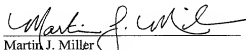
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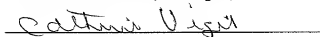
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NOVEL VITAMIN D RECEPTOR RELATED POLYPEPTIDES, NUCLEIC ACID SEQUENCE ENCODING THE SAME AND USES THEREOF

5 FIELD OF THE INVENTION

The present invention relates to novel vitamin D receptor related (VDRR) polypeptides. Nucleic acid sequences encoding the same, expression vectors containing such sequences and host cells transformed with such expression vectors are also disclosed, as are
10 methods for the expression of the novel VDRR polypeptides of the invention, and uses thereof.

BACKGROUND OF THE INVENTION

15 Nuclear hormone receptors is a large group of conditionally regulated transcription factors. These receptors are activated and regulate target gene expression in response to binding a variety of small chemical molecules (ligands) including steroids, vitamin D3, retinoids, eicosanoids (prostanoids), thyroid hormone and cholesterol derivatives.

A growing number of structurally related receptors have been identified for which no
20 ligands yet have been identified. This group of receptors is referred to as orphan nuclear receptors (ONRs). A review of the ONRs can be found in Enmark et al, Mol. Endo., vol. 10, No. 11 (1996) pp. 1293-1307, which is hereby incorporated by reference. The pivotal importance of a number of ONRs for processes such as metabolic homeostasis, cell differentiation and development have been demonstrated both by biochemical and genetic
25 techniques. In addition, several ONRs have also been implicated as key factors in a variety of common diseases and disorders such as diabetes, obesity, inflammatory conditions and proliferative diseases.

Based on these findings it is generally believed that novel ONRs are going to become potential drug targets for therapeutic invention of common diseases. Thus, it is of great
30 importance to identify such receptors.

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SUMMARY OF THE INVENTION

The present invention relates to novel vitamin D receptor related (VDRR) polypeptides, and formulations containing the same. Nucleic acid sequences encoding the VDRR polypeptides, expression vectors containing such sequences and host cells transformed with such expression vectors are also disclosed, as are methods for the expression of the novel VDRR polypeptides of the invention. The invention further relates to VDRR polypeptides for use as medicaments, and use of substances affecting VDRR signal transduction for the manufacture of medicaments for treating metabolic, proliferative or inflammatory conditions. The present invention also relates to methods for identifying clones encoding a VDRR polypeptide, methods for identifying ligands to a VDRR and methods for identifying substances for treatment of conditions affected by a VDRR polypeptide. More specifically, the novel VDRR polypeptide can be the polypeptide designated VDRR γ , which may be regulated by any small chemical molecule similar in structure to known ligands for nuclear receptors.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 - The cDNA sequence encoding the novel nuclear receptor polypeptide vitamin D receptor related gamma (VDRR γ) is shown.

Figure 2 - Evolutionary neighbor-joining tree for VDRR γ as given by DBD-HMM alignment.

Figure 3 - Evolutionary neighbor-joining tree for VDRR γ as given by LBD-HMM alignment.

Figure 4 - The deduced amino acid sequence of VDRR γ is shown.

Figure 5 - Expression of VDRR γ in adult human tissues. The numbers on the right hand side, refer to kilobasepairs of the mRNA.

Figure 6 - Vitamin D3 transactivate a GAL4-DBD/VDR-LBD fusion protein but not a GAL4-DBD/VDRR γ -LBD fusion protein in transient transfections of CV-1 cells. The number on the left hand side refer to relative luciferase activity of the GAL4-luciferase reporter gene.

Figure 7 - The cDNA sequence encoding VDRRg-2 with an alternatively spliced 5'-end compared to VDRRg is shown.

Figure 8 - The deduced amino acid sequence of VDRRg-2 is shown.

Figure 9 - Heterodimerization of VDRRg with a retinoid X receptor (RXR) is shown.

Figure 10 - The effect of pregnenolone derivatives as activators of VDRRg are shown.

Figure 11 - The effect of pregnenolone 16 α -carbonitrile (PCN), dexamethasone and an antiprogesterin (RU486) as activators of VDRRg are shown.

Figure 12 - Percent similarity between the new genes VDRRg-1 and VDRRg-2 and the known genes XOR-6, HVDR, CAR-1 and CAR-2.

Figure 13 - Percent identity between the new genes VDRRg-1 and VDRRg-2 and the known genes XOR-6, HVDR, CAR-1 and CAR-2.

DETAILED DESCRIPTION OF THE INVENTION

The objects above are met by the present invention, which relates to a mammalian, preferably human, isolated or recombinant nucleic acid comprising a contiguous nucleic acid sequence encoding a vitamin D receptor related (VDRR) polypeptide. The VDRR polypeptide is suitably origin.

In preferred embodiments of the present invention, the nucleic acid encoding the VDRR polypeptide contains a DNA-binding domain (DBD) comprising about 77 amino acids with 9 cysteine residues. The DBD is further characterized by the following amino acid sequence similarity relative to the DBDs of human Vitamin D Receptor (hVDR) and Orphan Nuclear Receptor 1 isolated from *Xenopus laevis* (xONR1 = XOR-6), respectively:

- (i) at least about 60% amino acid sequence similarity with the DBD of hVDR; and
- (ii) at least about 65% amino acid sequence similarity with the DBD of xONR1.

More particularly, the amino acid sequence similarity relative to the DBDs of hVDR and xONR1, respectively is

- (i) about 65% amino acid sequence similarity with the DBD of hVDR; and

(ii) about 71% amino acid sequence similarity with the DBD of xONR1.

In preferred embodiments of the present invention, the nucleic acid encoding the VDRR polypeptide contains a ligand-binding domain (LBD) characterized by the following amino acid sequence similarity, relative to the LBDs of hVDR and xONR1, respectively:

- 5 (i) at least about 30% amino acid sequence similarity with the LBD of hVDR, suitably at least 35% amino acid sequence similarity with the LBD of hVDR; and
- (ii) at least about 40% amino acid sequence similarity with the LBD of xONR1, suitably at least 45% amino acid sequence similarity with the LBD of xONR1.

More particularly, the amino acid sequence similarity relative to the LBDs of hVDR and xONR1, respectively is

- 10 (i) about 42% amino acid sequence similarity with the LBD of hVDR; and
- (ii) about 54% amino acid sequence similarity with the LBD of xONR1.

"amino acid sequence similarity" refers to: $100 \times \text{Consensus Length} / (\text{Consensus Length} + \text{Mismatches} + \text{Gaps})$.

15 The term amino acid sequence identity can also be used. Amino acid sequence identity is calculated by comparing the absolute amino acid residue identity. In Figure 13 the amino acid sequence identity between the new genes VDRRg-1 and VDRRg-2 and the known genes are shown.

In particularly preferred embodiments, the nucleic acid sequences of the present invention are substantially the same as those given in Fig. 1 or Fig. 7, the same or alleles thereof.

The present invention also relates to a nucleic acid probe for the detection of a nucleic acid sequence encoding a VDRR polypeptide in a sample. Suitably, the probe comprises at least 14 contiguous nucleotides, and preferably at least 28 contiguous nucleotides, of the nucleic acid sequences given in Fig. 1 or Fig. 7. The nucleic acid probe can be used in a method for identifying clones encoding a VDRR polypeptide, wherein the method comprises screening a genomic or cDNA library with the probe under low stringency hybridization conditions, and identifying those clones which display a substantial degree of hybridization to said probe.

30 The present invention further relates to an isolated or recombinant VDRR polypeptide. The polypeptide can be full-length, at which the sequence of amino acids is identical to

the corresponding sequence found in mammals in general, and in human beings in particular. In the present invention, the polypeptide can also be a truncated, extended or mutated form of the full-length polypeptide. Truncated and extended forms relate to VDRR polypeptides where one or more amino acids are missing or have been added, respectively, at the N terminal end of the polypeptide chain. Mutated forms relate to VDRR polypeptides where one or more amino acid has been substituted by another amino acid. Suitably, the isolated or recombinant VDRR polypeptide exhibits the amino acid sequences given in Fig. 4 or Fig. 8.

The N-terminal sequence of the present nucleic acids encoding VDRR polypeptides, as well as the amino acid sequence of the present VDRR polypeptides, may vary. Thus, various N-terminal isoforms are envisaged, e.g. any of $\alpha 1$, $\alpha 2$, $\beta 1$, $\beta 2$, $\beta 3$, $\beta 4$, $\gamma 1$ or $\gamma 2$ as disclosed in Fig. 7B of Transcription Factors 3: nuclear receptors, Protein Profile, vol. 2, issue 11 (1995), pp. 1173-1235. This review of nuclear receptors generally is hereby incorporated by reference. More specifically, Vitamin D receptors and related orphans, e.g. ONR1, are discussed at p. 1191-1992.

The present invention further relates to pharmaceutical formulations comprising an isolated or recombinant VDRR polypeptide, and one or more therapeutically acceptable excipients. Examples of excipients that can be used are carbohydrates, e.g. monosaccharides, disaccharides and sugar alcohols, such as saccharose and sorbitol. Further examples include amino acids, e.g. histidine and arginine, surfactants, e.g. polyoxyethylene sorbitan fatty acid esters, inorganic salts, e.g. sodium chloride and calcium chloride, and complexing agents, e.g. EDTA and citric acid.

The present formulation can be in the form of an aqueous solution ready-for-use, or dried, particularly lyophilized. In the latter case, the formulation is reconstituted with a liquid, e.g. sterile water or saline, before use.

The present invention further relates to an expression vector comprising an isolated or recombinant nucleic acid, the nucleic acid comprising a contiguous nucleic acid sequence encoding a Vitamin D receptor related (VDRR) polypeptide. The invention also relates to a cell containing such an expression vector.

The present invention further relates to a cell containing the claimed nucleic acid, the nucleic acid comprising a contiguous nucleic acid sequence encoding a Vitamin D receptor related (VDRR) polypeptide.

5 The present invention further relates to a process for recombinant production of a VDRR polypeptide, by expressing the claimed isolated or recombinant contiguous nucleic acid sequence encoding a Vitamin D receptor related (VDRR) polypeptide in a suitable host cell, preferably an eukaryotic cell.

10 The present invention further relates to method for identifying a ligand to a VDRR, e.g. by a cell-based reporter assay, transgenic-animal reporter assay or *in vitro*-binding assay. It also relates to a method for identifying a substance for treatment of a condition affected by a VDRR polypeptide, comprising screening for an agonist or an antagonist of VDRR polypeptide signal transduction to be used for treating metabolic, proliferative or inflammatory conditions.

15 The present invention further relates to a VDRR polypeptide for use as a medication, as well as use of a substance affecting VDRR signal transduction for the manufacture of a medicament for treating metabolic, proliferative or inflammatory conditions. More particularly, the present invention can be used for the manufacture of medicaments for treating obesity, diabetes, anorexia, lipoprotein defects, hyperlipidemia, hypercholesteremia or hyperlipoproteinemia. The present invention can be used also for the manufacture of
20 medicaments for treating osteoporosis, rheumatoid arthritis, benign and malign tumors, hyperproliferative skin disorders or hyperparathyroidism.

The present invention further relates to a method for treating metabolic, proliferative or inflammatory conditions by introducing into a mammal a nucleic acid vector encoding for expression of a VDRR polypeptide. The nucleic acid vector is capable of transforming a
25 cell *in vivo* and expressing said polypeptide in said transformed cell.

The present invention further relates to a method for treatment of a metabolic, proliferative or inflammatory condition by administration of a therapeutically effective amount of a substance affecting VDRR signal transduction, specifically a VDRR polypeptide.

30 In the present invention, the term "isolated" in connection with VDRR polypeptides or nucleic acids encoding the same, relates to nucleic acids or polypeptides that have been isolated from a natural source, e.g. the liver, small intestine or colon of a human being. The

isolated VDRR polypeptides or nucleic acids of the present invention are unique in the sense that they are not found in a pure or separated form in nature. Use of the term "isolated" indicates that a naturally occurring sequence has been removed from its normal cellular environment. Thus, the sequence may be in a cell-free environment or in a different cellular environment. The term does not imply that the sequence is the only nucleic acid or amino acid sequence present, but that it is the predominant nucleic acid or amino acid sequence present. Furthermore, the nucleic acid or polypeptide should be essentially free of non-amino acid or non-nucleic acid material naturally associated with the respective product. In this context, essentially free relates to more than 80%, suitably more than 90%, and preferably more than 95% purity.

The term "substantially the same" when referring to the nucleic acid sequences in Fig 1 or Fig 7 and when referring to the amino acid sequences in Fig. 4 or Fig. 8 means that they are derived from the sequences given in the figures and have the same function as those.

The inventors of the present invention, have surprisingly isolated a novel nucleic acid sequence, and a polypeptide encoded by said nucleic acid sequence. Thus, a novel cDNA encoding a polypeptide designated VDRR γ has been cloned and characterized. This polypeptide is, based on amino acid sequence similarity, a novel member of the nuclear (hormone) receptor supergene family. Hidden Markov Models (HMMs) in combination with phylogenetic analysis such as neighbor-joining tree methods and other statistical algorithms shows that VDRR γ belong to a sub-family of vitamin D receptors (VDRs) and a VDR-like receptor from *Xenopus laevis* designated xONR1 (see Smith et al., Nucl. Acids Res., 22 (1994), No. 1, pp. 66-71) or XOR-6 as in WO96/22390. The VDRR γ , therefore, is one member of a family of Vitamin D receptor related (VDRR) polypeptides.

The degree of amino acid similarity in the DBD and LBD of VDRR γ as compared to the most closely related receptors XOR-6, hVDR and CAR (see WO 93/17041) is similar to the relationship between other distinct, but related nuclear receptors. (See Fig. 12). The thyroid hormone (TRb) and retinoic acid receptor (RARb) are approximately 60% and 40% identical at the amino acid level in the DBD and LBD, respectively. By comparison, the closely related but unique genes encoding human RAR α and RAR β nuclear receptors are 97% and 82% identical in the DBD and LBD, respectively.

As recognized by those skilled in the art of nuclear receptors, the DBD displays the highest degree of conservation (amino acid identity) both between different nuclear receptors (paralogous) and between identical receptors from different species (orthologues). The two "zink-fingers" in the DBD are generated by two evolutionary conserved amino acid motifs Cys-X2-Cys-X13-Cys-X2-Cys (amino-terminal or first zink-finger) and Cys-Xn-Cys-X9-Cys-X2-Cys (carboxy-terminal or second zink-finger) in which two pairs of cysteins chelate on zink ion. The vast majority of nuclear receptors have five amino acid residues between the first two Cys residues in the second zink-finger (Cys-X5-Cys-X9-Cys-X2-Cys) see Gronemeyer and Laudet (Protein Profile 1995, 2, issue 11) for details. The today only known exception to this rule are the PPARs which have three amino acid (Cys-X3-Cys-X9-Cys-X2-Cys) residues and the TLL group of receptors which have seven (Cys-X7-Cys-X9-Cys-X2-Cys). Thus another feature which is characteristic of the novel VDRRg polypeptide described herein is that the number of amino acid residues in this part of the DBD is six (Cys-X6-Cys-X9-Cys-X2-Cys) as shown in Figs.4 and 8. Today, the only other nuclear receptor like sequences found in the TREMBLE data base with the same number of amino acid residues between the two cys residues are two sequences (Q20097 and Q18155) from the worm *C. elegans* (Q20097 and Q18155). However, the entire DBD of these putative *C. elegans* nuclear receptors are only distantly related to the DBD of VDRRg. Taken together, the comparison of the DBD and LBD of the nuclear receptor VDRRg described herein (See Fig.12), clearly demonstrate that this receptor is a novel member of the nuclear receptor super-gene family which is distinct from other known nuclear receptors that are most closely related to the VDRRg including ONR-1 (in Smith et al., 1994, Nucleic Acids Res., 22, pp66-71) or XOR-6 (in WO 96/22390), hVDR and CAR (WO 93/17041).

This finding, in combination with the highly restricted expression pattern we observe for human VDRRγ (liver, small intestine and mucosa of colon) and in analogy to other nuclear receptors exhibiting a tissue specific expression pattern such as the peroxisome proliferator-activated receptors (PPARs) - suggest that VDRRγ performs important physiological functions in liver, small intestine and colon. Accordingly, VDRRγ is likely to be an important sensor of key metabolic pathways affecting lipid, carbohydrate or amino acid metabolism/homeostasis. In addition, the highly selective tissue specific expression pattern

suggest that VDRR γ may participate in cellular differentiation and development of these tissues.

An additional human VDRR γ cDNA with an alternatively spliced 5'-end has been identified (see Fig. 7). The VDRR γ cDNAs are thus able to encode at least one alternative N-terminal variant (Fig. 8) in addition to the VDRR γ polypeptide shown in Fig. 4. In analogy to other members of the nuclear receptor supergene family such as ROR α and RAR α these N-terminal isoforms of VDRR γ may specify different functions including DNA-binding specificity and/or promoter specific activation (Gronemeyer and Laudet, 1995).

In the present specification, the term VDRR γ relates to the various polypeptides corresponding to the differentially spliced VDRR γ cDNAs including VDRR γ -1 and VDRR γ -2. However, when reference is made to Fig. 1 and Fig. 4, VDRR γ cDNA and VDRR γ relates specifically to VDRR γ -1 cDNA and VDRR γ -1, respectively. In the same way, when reference is made to Fig. 7 and Fig. 8, VDRR γ cDNA and VDRR γ relates specifically to VDRR γ -2 cDNA and VDRR γ -2, respectively.

In contrast to the VDRR γ -2 cDNA, the VDRR γ -1 cDNA does not contain a classical AUG initiation codon but instead may initiate at an alternative CUG codon. This putative non-AUG start site is located in a favorable sequence context for efficient initiation from alternative start sites and is in frame with the entire open reading frame and preceded by a stop codon.

Taken together, the VDRRs in general, and more specifically the VDRR γ , may be important in

- 1) metabolic diseases such as obesity, diabetes (type I and II), lipoprotein disorders,
- 2) proliferative conditions such as tumors (benign and malignant) of the small intestine and colon,
- 3) ulcero-inflammatory diseases of small intestine and colon such as Crohn's disease and ulcerative colitis, and
- 4) congenital anomalies of small intestine and colon.

The high amino acid sequence identity of VDRR γ with the VDR both in the DNA-binding domain (DBD) and ligand-binding domain (LBD) indicate that these two receptors

may also have overlapping yet distinct functional characteristics. In analogy, retinoic acid receptors (RARs) and retinoid X receptors (RXRs) have similar amino acid sequence identities in the DBD and LBD region as the VDR and VDRR γ . RARs and RXRs have been shown to have distinct functional similarities such that both receptors bind 9-cis retinoic acid and have overlapping DNA-binding specificities and accordingly regulate overlapping gene networks. Based on these findings, VDRR γ may be regulated by small chemical molecules similar in structure to known ligands for nuclear receptors but not necessarily identical to ligands for the 1 α , 25-dihydroxy vitamin D3 receptor. Furthermore, VDRR γ may regulate vitamin D3 responsive gene networks by binding to a Vitamin D responsive element (VDRE)-like DNA sequence. In the present application, the 1 α , 25-dihydroxy vitamin D3 receptor is abbreviated as the Vitamin D receptor (VDR).

In the present invention, the substance affecting VDRR signal transduction can be any small chemical molecule of natural or synthetic origin, e.g. a carbohydrate such as an aromatic compound. The small molecule may have a molecular weight in the range of from about 100 up to about 500 Da. Suitably, the small chemical molecule has a molecular weight in the range of from 200 up to 400 Da. Preferably, the small chemical molecule has a molecular weight of about 300 Da.

The human VDRR γ polypeptides, including VDRR γ -1 and VDRR γ -2, have been shown to be activated e.g. by pregnenolones and estradiol (weakly), but not by certain other steroid hormones such as cortisol, aldosterone, progesterone and estrogen, and most likely not by progestins and glucocorticoids. Thus, human VDRR γ is not activated by pregnenolone 16 α -carbonitrile (PCN), a glucocorticoid antagonist. For this reason, human VDRR γ can also be designated human pregnenolone activated (nuclear) receptors (hPAR). Information about pregnenolone can be found e.g. in the Merck Index, 11th ed., Merck & Co., Inc. Rahway, N.J., USA, p. 7735, 1989.

Activators for human VDRR γ polypeptides, including VDRR γ -1 and VDRR γ -2, (hPAR-1 and hPAR-2, respectively), include but are not limited to pregnenolones, such as pregnane-ones, pregnane-diones, pregnane-triones, and pregnane-diols, and androstanes, such as androstane-ols, and androstane-diols. Suitably, the pregnenolones are non-planar, particularly 5 β -pregnanes.

Specific examples of activators and possibly ligands for human VDR γ polypeptides, including VDR γ -1 and VDR γ -2, are the following compounds, which are marketed by Sigma-Aldrich of Sweden:

- i) 5 β -pregnane-3,20-dione
- 5 ii) 3 α -hydroxy-5 β -pregnane-11,20-dione methanesulphonate
- iii) 5 β -pregnane-3 α ,20 β -diol
- iv) pregnenolone
- v) Pregn-4-eno[16,17-8][2]isoxazoline-3,20-dione, 6 α -methyl-3'-phenyl-, ethyl ether solvate
- 10 vi) Pregna-1,4,9(11)-triene-3,20-dione, 21-[4-[6-methoxy-2-(4-morpholinyl)-4-pyrimidinyl]-1-piperazinyl]-16-methyl-, (16 α)-
- vii) Estran-3-ol, 17-[[[3-(trifluoromethyl)phenyl]methyl]amino]-, (E)-2-butenedioate (1:1) (salt)
- viii) 9 α -Fluoro-5 α -androstane-11 β ,17 β -diol
- 15 ix) Spiro[5 α -androstane-3,2'-benzothiazolin]-11-one, 17 β -hydroxy-17-methyl-
- x) Spiro[pregnane-3,2'-thiazolidine]-4'-carboxylic acid, 11 α -hydroxy-20-oxo-, sodium salt
- xi) 17 β -Dimethylamino-17-ethynyl-5 α -androstane-11 β -ol
- xii) 6 β -Hydroxy-3,5-cyclo-5 α -pregnan-20-one, nitrite
- 20 xiii) 3 α -Hydroxy-5 β -pregnane-11,20-dione, acetate, 20-O-(methylsulfonyl)-oxime
- xiv) 17 α -Methyl-5 α -androstane-11 β ,17-diol
- xv) 5 β -Pregnane-3,11,20-trione, trioxime
- xvi) 3 α -Hydroxy-5 β -pregnane-11,20-dione, 20-hydazone with hydrazide of 1-(carboxymethyl) pyridinium chloride.
- 25 A possible use of a VDR γ antagonist, could be a synergistic co-administration of the VDR γ antagonist together with other drugs such as, but not limited to, HIV protease inhibitors and cyclosporin to inhibit the expression of CYP3A4 and thus increase the bioavailability of drugs with poor pharmacokinetics due to CYP3A4 metabolism. Genes coding for polypeptides, such as human vitamin D receptor related gamma
- 30 (hVDR γ), may be cloned by incorporating a DNA fragment coding for the polypeptide into a recombinant DNA vehicle, e.g. a vector, and transforming suitable prokaryotic or

eukaryotic host cells. Such recombinant DNA techniques are well known and e.g. described in *Methods in Enzymology*, Academic Press, San Diego, CA, USA (1994), vols. 65 and 68 (1979), and vols. 100 and 101 (1983).

The host cells for use in the present invention can be prokaryotic or eukaryotic, preferably eukaryotic cells. Suitable eukaryotic host cells include but are not limited to cells from yeast, e.g. Saccharomyces, insect cells and mammalian cells such as Chinese Hamster Ovary (CHO), Baby Hamster Kidney (BHK), COS and the like. Suitable prokaryotic host cells include but are not limited to cells from Enterobacteriaceae, e.g. E. coli, Bacillus and Streptomyces.

EXAMPLES

The following Examples are provided for purposes of illustration only and are not to be construed as in any way limiting the scope of the present invention, which is defined by the appended claims.

EXAMPLE 1

Identification and isolation of human VDRRg cDNA

Expressed Sequence Tag (EST) databases were screened for nuclear receptor related sequences with a DNA-binding domain (DBD) profile of nuclear receptors. This search profile was created by multiple alignment of a selected set of nuclear receptor sub-domains followed by a statistical calculation to obtain a so called Hidden Markov Model (HMM) of different subfamily members of the nuclear receptor supergene family. The cDNA of one of the nuclear receptor related EST sequences identified (Incyte clone no 2211526) was analyzed in detail by sequencing. After DNA sequencing of the entire Incyte cDNA clone (approximately 2200 basepairs) the clone was found to encode a putative ligand-binding domain (LBD) with 54% and 44% similarity to xONR-1 and to the vitamin D receptor (VDR), respectively. The cDNA of the Incyte clone was not full-length and did not encode a sequence corresponding to a complete DBD.

5'-RACE (rapid amplification of cDNA ends) of random primed cDNA from human liver RNA (Invitrogen) followed by cloning and DNA sequencing showed that the 5'-part of the cDNA corresponding to the Incyte clone encoded a DBD characteristic for nuclear receptors and with 71% and 65% sequence similarity to xONR-1 and VDR, respectively.

Multiple alignments in combination with evolutionary neighbor-joining tree analysis placed the polypeptide encoded by the cDNA (specified in Fig. 1) in the group of VDRs (Figs. 2 and 3) and was named human vitamin D receptor related gamma (VDRR γ). The deduced amino acid sequence of VDRR γ is given in Fig. 4.

EXAMPLE 2

Expression of VDRR γ mRNA in human tissues

Multiple tissue northern blots (Clontech) was used to determine the expression pattern of VDRR γ in adult human tissues. As shown in Fig. 5, VDRR γ is abundantly expressed in small intestine, mucosal lining of colon and liver but not in several other tissues including spleen, thymus, prostate, testis, ovary, peripheral blood leukocytes, heart, brain, placenta, lung, skeletal muscle, kidney and pancreas. To investigate if VDRR γ was expressed at lower levels in any of the other tissues examined, the filter was exposed for an extended time (one week as compared to overnight). Even after this prolonged exposure (data not shown), expression could still only be detected in the same tissues and not in any of the other tissues examined. The restricted expression pattern of VDRR γ suggest that this receptor is likely to have an important regulatory function in liver and intestine.

EXAMPLE 3

Transient transfections of GAL4-DBD/VDRR γ -LBD fusion protein using Vitamin D3

Transient transfections were performed to analyze if vitamin D3 activate the VDRR γ polypeptide. To this end, transient co-transfections of CV-1 cells were performed with expression plasmids encoding fusion proteins of the GAL4-DBD fused to the LBD of either the VDR or the VDRR together with a reporter-plasmid containing five GAL4 responsive elements upstream of the luciferase gene. After transfection, cells were treated with vehicle (DMSO) alone or with vitamin D3 for 48 hours followed by harvesting of the cells and measurement of the luciferase activity in cell extracts. As shown in Fig. 6, vitamin D3 (1

μ M) transactivate the GAL4-DBD/VDR-LBD but not the corresponding GAL4-DBD/VDR γ -LBD polypeptide under these conditions. This indicates that the two receptors may have distinct ligand-binding specificities.

5 EXAMPLE 4

Identification and isolation of human VDR γ cDNAs encoding multiple N-terminal isoforms

5'-RACE (see Example 1) of cDNA from human liver RNA followed by cloning and DNA sequencing identified an additional human VDR γ cDNA with alternatively spliced 5'-end (see Fig. 7). The VDR γ cDNAs are thus able to encode at least one alternative N-terminal variant (Fig. 8) in addition to the VDR γ polypeptide shown in Fig. 4. The polypeptides disclosed in Fig. 4 and Fig. 8 which correspond to the differentially spliced VDR γ cDNAs are designated as VDR γ -1 and VDR γ -2, respectively.

15 EXAMPLE 5

VDR γ heterodimerise with RXR and bind to direct repeats (DRs) spaced by three or four nucleotides

Expression plasmids containing VDR γ or RXR β cDNAs were transcribed using T7 polymerase and translated *in vitro* in TNT reticulocyte lysates (Promega, Madison, WI, USA). To investigate the DNA-binding specificity of VDR γ a native gel mobility assay was employed essentially as described (Berkenstam et al., Cell, 69, 401-412, 1992) in which *in vitro* translated VDR γ was incubated in the presence or absence of *in vitro* translated RXR β with different 32P-labelled direct repeats (DR-1 to DR-5) as indicated in Fig. 9. The direct repeats were derived from the DR-5 element in the RAR- β 2 promoter (de Thé et al., Nature, 343, 177-180, 1990) and modified to be separated by one to five nucleotides (Pettersson et al., Mechanisms of Dev., 54, 1-13, 1995). Protein-DNA complexes were separated on native 5% polyacryl-amide/0.25xTBE gels followed by autoradiography. As shown in Fig. 9, of the five DRs tested efficient VDR γ binding could only be detected with DRs separated by three or four nucleotides and only in the presence of RXR. However, weaker RXR-dependent binding could also be observed to DR-2 and DR-1 elements. These

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results demonstrate that VDR γ require RXR heterodimerisation for efficient DNA-binding to a specific subset of DRs. These results, however, do not exclude the possibility that VDR γ may bind as a monomer, dimer or heterodimer to distinct but related DNA-sequences. Importantly, our results demonstrate that VDR γ and other nuclear receptors

- 5 including the VDR (e.g. Markose, E. R. et al., Proc. Natl. Acad. Sci. USA, 87, 1701-1705, 1990), THR α s (e.g. Gronemeyer, H. and Moras, D., Nature, 375, 190-191, 1995), LXR α s (e.g. Willy, P. J. et al., Genes. Dev., 9, 1033-1045, 1995), have distinct but overlapping DNA-sequence and thus may regulate overlapping gene networks.

- 10 Interestingly, the most closely related nuclear receptor called ONR-1 (in Smith et al., 1994, Nucleic Acids Res., 22, pp66-71) or XOR-6 (in WO 96/22390) have been reported to "bind well to a retinoic acid response element, bRARE" (p. 11, line 30 in WO 96/22390). However, although the novel nuclear receptor VDR γ reported herein has 71% amino acid similarity in the DBD as compared to XOR-6 (fig 12), VDR γ does not appear to bind to the same bRARE sequence (DR-5 in Fig. 9).

15

EXAMPLE 6

Pregnenolone derivatives as activators of VDR γ

For identifying activators or ligands for VDR γ , a library of substances structurally biased towards different classes of activators and ligands for nuclear receptors were tested.

- 20 The activation of VDR γ was analyzed in a reporter gene assay in transiently Caco-2 (TC7) cells (Carriere et al, 1994). In this initial screen, the synthetic substances with ability to activate VDR γ were found to be structurally similar to pregnenolones (data not shown). Based on these results, naturally occurring pregnenolone derivatives were examined for activation of VDR γ . The results are shown in Fig. 10. As is evident from Fig. 10, VDR γ
- 25 was activated about 5 to 12 fold by pregnenolone, 5 β -pregnane-3,20-dione, 5 β -pregnane-3 α ,20 β -diol and 3 α -hydroxy-5 β -pregnane-11,20-dione methanesulphonate. In contrast to the efficient activation observed by the 5 β -pregnane-3,20-dione, the corresponding planar steroid derivative 5 α -pregnane-3,20-dione did not activate the receptor. Other 5 β -pregnanes also activated VDR γ efficiently as opposed to all planar pregnenolone derivatives tested, as
- 30 is also evident from Fig. 10.

EXAMPLE 7

Pregnenolone 16 α -carbonitrile (PCN), dexamethasone and an antiprogesterin (RU486) as activators of VDRRg

- 5 Further experiments were performed to find out if pregnenolone 16 α -carbonitrile (PCN), a glucocorticoid antagonist or dexamethasone are activators of VDRR γ . To this effect, Caco-2 cells were transfected as before with VDRR γ and the activation was analyzed after treatment of the cells with 10 μ M PCN or dexamethasone. The results are shown in Fig. 11. As is evident from Fig. 11, VDRR γ was not activated by these substances, indicating that VDRR γ is not the human PCN receptor. This suggestion is corroborated by the observation that also the antiprogesterin RU486 only caused a slight increase (two fold) in VDRR γ mediated reporter gene activity as is evident from Fig. 11.
- 10 Activators of XOR-6 (Fig. 3 in WO 96/22390) such as butyl 4-NH₂ Benzoate did not activate VDRRg (data not shown) in similar reporter assays as used in WO 96/22390.

CLAIMS

5

1. A mammalian, preferably human, isolated or recombinant nucleic acid comprising a contiguous nucleic acid sequence encoding a vitamin D receptor related (VDRR) polypeptide.

10

2. An isolated or recombinant DNA/nucleic acid according to Fig. 1 or Fig. 7 or alleles thereof encoding a new VDRR polypeptide.

15

3. The nucleic acid according to claim 1 or claim 2 encoding the VDRR polypeptide containing a DNA-binding domain (DBD) comprising about 77 amino acids with 9 cysteine residues,, wherein said DBD is characterized by the following amino acid sequence similarity:

20

- (i) at least 60% amino acid sequence similarity with the DBD of hVDR; and
- (ii) at least 65% amino acid sequence similarity with the DBD of xONR1.

25

4. The nucleic acid according to claim 3, wherein said DBD is characterized by the following amino acid sequence similarity:

- (i) about 65% amino acid sequence similarity with the DBD of hVDR; and
- (ii) about 71% amino acid sequence similarity with the DBD of xONR1.

30

5. The nucleic acid according to any previous claim, encoding the VDRR polypeptide, wherein the ligand-binding domain (LBD) of said polypeptide is characterized by the following amino acid sequence similarity, relative to the LBDs of hVDR and xONR1, respectively:

- (i) at least about 30% amino acid sequence similarity with the LBD of hVDR; and
- (ii) at least about 40% amino acid sequence similarity with the LBD of xONR1.

6. The nucleic acid according to claim 5, wherein said LBD is characterized by the following amino acid sequence similarity:

- (i) at least 35% amino acid sequence similarity with the LBD of hVDR; and
- (ii) at least 45% amino acid sequence similarity with the LBD of xONR1.

7. The nucleic acid according to claim 6, wherein said LBD is characterized by the following amino acid sequence similarity:

- (i) about 42% amino acid sequence similarity with the LBD of hVDR; and
- (ii) about 54% amino acid sequence similarity with the LBD of xONR1.

8. The nucleic acid according to any previous claim, wherein said nucleic acid sequence is that given in Fig. 1 or Fig. 7 or alleles thereof.

9. The nucleic acid according to claim 8, wherein said nucleic acid sequence is the same or substantially the same as given in Fig. 1 or Fig. 7.

10. A nucleic acid probe for the detection of a nucleic acid sequence encoding a VDRR polypeptide in a sample.

11. The nucleic acid probe according to claim 10, wherein said probe comprises at least 14 contiguous nucleotides of the nucleic acid sequence given in Fig. 1 or Fig. 7.

12. A method for identifying clones encoding a VDRR polypeptide said method comprising screening a genomic or cDNA library with a nucleic acid probe according to claim 10 or 11 under low stringency hybridization conditions, and identifying those clones which display a substantial degree of hybridization to said probe.

13. An expression vector comprising a nucleic acid according to any of claims 1 -9.

14. A cell containing a nucleic acid according to any of claims 1 -9.

15. A cell containing an expression vector according to claim 14.

16. A process for recombinant production of a VDRR polypeptide, said process comprising
5 expressing the nucleic acid of any of claims 1 to 9 in a suitable host cell.

17. The process according to claim 16, wherein the host cell is eukaryotic.

18. An isolated or recombinant mammalian, preferably human, VDRR polypeptide.

10

19. The isolated or recombinant VDRR polypeptide according to claim 18 comprising the
amino acid sequence substantially the same or the same as given in Fig. 4 or Fig. 8.

15

20. A method to produce specific monoclonal and polyclonal antibodies to the polypeptide
according to any of claims 18 and 19 comprising the injection of the protein to a
mammalian.

20

21. A pharmaceutical formulation comprising an isolated or recombinant VDRR
polypeptide according to any of claim 18 and 19, and one or more therapeutically
acceptable excipients.

22. A method for identifying a ligand to a VDRR according to any of claim 18 and 19, by a
cell-based reporter assay, transgenic-animal reporter assay or *in vitro*-binding assay.

25

23. A method for identifying a substance for treatment of a condition affected by a VDRR
polypeptide according to any of claim 18 and 19, comprising screening for an agonist or
an antagonist of VDRR polypeptide signal transduction to be used for treating metabolic,
proliferative or inflammatory conditions.

30

24. A mammalian, preferably human, VDRR polypeptide according to any of claim 18 and
19 for use as a medicament.

25. Use of a substance affecting VDRR, according to any of claim 18 and 19, signal transduction, such as an agonist or an antagonist of VDRR polypeptide signal transduction, for the manufacture of a medicament for treating metabolic, proliferative or inflammatory conditions.

26. Use of a substance affecting VDRR, according to any of claim 18 and 19, signal transduction for the manufacture of a medicament for treating obesity, diabetes, anorexia, lipoprotein defects, hyperlipidemia, hypercholesteremia or hyperlipoproteinemia.

27. Use of a substance affecting VDRR, according to any of claim 18 and 19, signal transduction for the manufacture of a medicament for treating osteoporosis, rheumatoid arthritis, benign and malign tumors, hyperproliferative skin disorders or hyperparathyroidism.

28. Use according to any of claims 25-27, wherein the substance affecting VDRR signal transduction is a chemical molecule of natural or synthetic origin with a molecular weight in the range of from about 100 up to about 500 Da, preferably with a molecular weight of about 300 Da.

29. A method for treating metabolic, proliferative or inflammatory conditions comprising introducing into a mammal a nucleic acid vector according to claim 13 encoding for expression of a VDRR polypeptide and wherein said nucleic acid vector is capable of transforming a cell *in vivo* and expressing said polypeptide in said transformed cell.

30. A method for treatment of a metabolic, proliferative or inflammatory condition by administration of a therapeutically effective amount of a substance affecting VDRR, according to any of claim 18 and 19, signal transduction.

31. The method according to claim 30, wherein the substance affecting VDRR signal transduction is a chemical molecule of natural or synthetic origin with a molecular weight in the range of from about 100 up to about 500 Da, preferably with a molecular weight of about 300 Da.

5

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ABSTRACT

The present invention relates to novel vitamin D receptor related (VDRR) polypeptides, and formulations containing the same. Nucleic acid sequences encoding the VDRR polypeptides, expression vectors containing such sequences and host cells transformed with such expression vectors are also disclosed, as are methods for the expression of the novel VDRR polypeptides of the invention. The invention further relates to VDRR polypeptides for use as medicaments, and use of substances affecting VDRR signal transduction for the manufacture of medicaments for treating metabolic, proliferative or inflammatory conditions. The present invention also relates to methods for identifying clones encoding a VDRR polypeptide, methods for identifying ligands to a VDRR and methods for identifying substances for treatment of conditions affected by a VDRR polypeptide. More specifically, the novel VDRR polypeptide can be the polypeptide designated VDRR γ , which may be regulated by any small chemical molecule similar in structure to known ligands for nuclear receptors.

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1 CCTCTGAAGG TTCTAGAAATCATAGTGAATTCGTGGGACG GGAAGAGGAA
 51 GCACTGCCTTTACTTCAGTG GGAATCTCGG CCTCAGCCTG CAAGCCAAGT
 101 GTTCACAGTG AAAAAAGCAA GAGAATAAGC TAAATCTCCT GTCTGAACA
 151 AGGCAGCGCG TCCTTGGTAA AGCTACTCCT TGATCGATCC TITGCACCGG
 201 ATTGTTCAAA GTGGACCCCA GGGGAGAAGT CGGAGCAAAG AACTTACCAC
 251 CAAGCAGTCC AAGAGGCCCA GAAGCAAAAC TGGAGGTGAG ACCCAAAGAA
 301 AGCTGGAACC ATGCTGACTT TGTACACTGT GAGGACACAG AGTCTGTTCC
 351 TGGAAAGCCC AGTGCAACG CAGATGAGGA AGTCGGAGGT CCCCAAATCT
 401 GCCGTGTATG TGGGGACAAG GCCACTGGCT ATCACTTCAA TGTCATGACA
 451 TGTGAAGGAT GCAAGGGCTT TTTCAGGAGG GCCATGAAAC GCAACGCCCC
 501 GCTGAGGTGC CCCTTCCGGG AGGGCGCCTG CGAGATCACC CGGAAGACCC
 551 GCGGACAGTG CCAGGCCTGC CGCTGCGCA AGTCCTTGA GAGCGGCATG
 601 AAGAAGGAGA TGATCATGTC CGACGAGGCC GTGGAGGAGA GCGGGGCCTT
 651 GATCAAGCGG AAGAAAAGTG AACGGACAGG GACTCAGCCA CTGGGAGTGC
 701 AGGGGCTGAC AGAGGAGCAG CGGATGATGA TCAGGGAGCT GATGGACGCT
 751 CAGATGAAAA CCTTTGACAC TACCTTCTCC CATTCAAGA ATTCCGGCT
 801 GCCAGGGGTG CTTAGCAGTG GCTGCGAGTT GCCAGAGTCT CTGCAGGCC
 851 CATCGAGGGA AGAAGCTGCC AAGTGGAGCC AGGTCCGGAA AGATCTGTGC
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 951 CTACAAACCC CCAGCCGACA GTGGCGGGAA AGAGATCTTC TCCCTGCTGC
 1001 CCCACATGGC TGACATGTCA ACCTACATGT TCAAAGGCAT CATCAGCTTT
 1051 GCCAAAGTCA TCTCCTACTT CAGGGACTTG CCCATCGAGG ACCAGATCTC
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 1151 TGTTCACGC GGAGACTGGA ACCTGGGAGT GTGGCCGGCT GTCCTACTGC
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Fig. 1

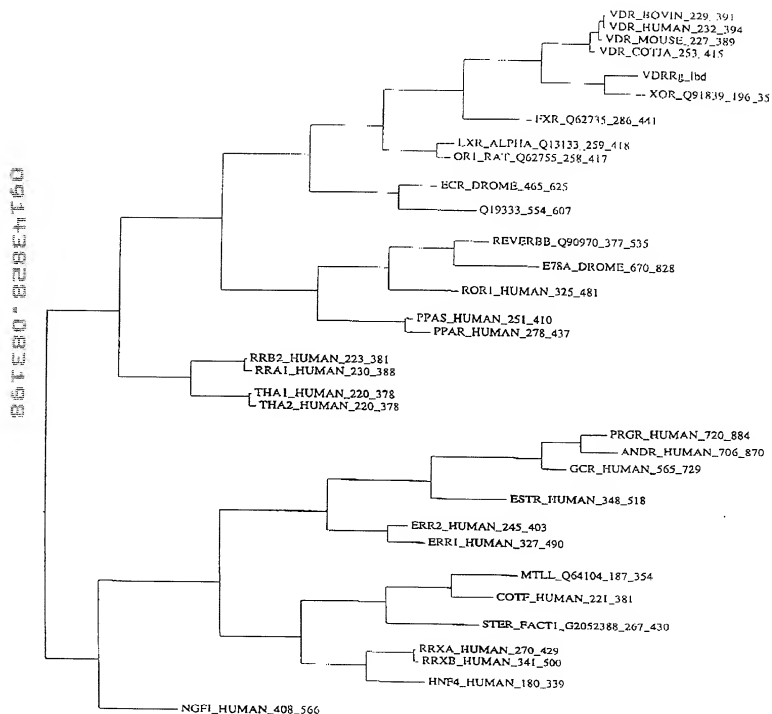
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 1501 ACCCAGCGGC TGCTGCGCAT CCAGGACATA CACCCCTTTG CTACGCCCCCT
 1551 CATGCAGGAG TTGTTGCGCA TCACAGGTAG CTGAGCGGCT GCCCTTGGGT
 1601 GACACCTCCG AGAGGCAGCC AGACCCAGAG CCTCTGAGC CGCCACTCCC
 1651 GGGCCAAGAC AGATGGACAC TGCCAAGAGC CGACAATGCC CTGCTGGCCT
 1701 GTCTCCCTAG GGAATTCCTG CTATGACAGC TGGETAGCAT TCCTCAGGAA
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 1801 AGACTCTTAC GTGGAGAGTG CACTGACCTG TAGGTCAGGA CCATCAGAGA
 1851 GGCAAGGTTG CCCTTTCCCT TTAAGAGGCC CTGTGGTCTG GGGAGAAATC
 1901 CCTCAGATCC CACTAAAGTG TCAAGGTGTG GAAGGGACCA AGCGACCAAG
 1951 GATAGCCCAT CTGGGGTCTA TGCCACATA CCCACGTITG TTCGCTCCT
 2001 GAGTCTTTTC ATTGCTACCT CTAATAGTCC TGTCTCCAC TTCCCCTCG
 2051 TTCCCTCCT CTTCGAGCT GCTTTGTGGG CTCAAGGCCT GTACTCATCG
 2101 GCAGGTGCAT GAGTATCTGT GGGAGTCTC TAGAGAGATG AGAAGCCAGG
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 2201 CATTCTGTGT CTCTGCATCC ATTTGAACAC ATTATTAAGC ACTGATAATA
 2251 GGTAGCCTGC TGTGGGGTAT ACAGCATTGA CTCAGATATA GATCTGAGC
 2301 TCACAGAGTT TATAGTTAAA AAAACAAACA GAAACACAAA CAATTTGGAT
 2351 CAAAAGGAGA AAATGATAAG TGACAAAAGC AGCACAAGGA ATTTCCCTGT
 2401 GTGGATGCTG AGCTGTGATG GCAGGCACTG GGTACCCAAG TGAAGGTTCC
 2451 CGAGGACATG AGTCTGTAGG AGCAAGGGCA CAAACTGCAG CTGTGAGTGC
 2501 GTGTGTGTGA TTTGGTGTAG GTAGGTCTGT TTGCCACTTG ATGGGGCCTG
 2551 GGTTTGTTC TGGGGCTGGA ATGCTGGGTA TGCTCTGTGA CAAGGCTACG
 2601 CTGACAAATCA GTTAAACACA CCGAGAAGA ACCATTTACA TGCACCTTAT
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 2701 CCTTGTTTAT AGCCACTTGT GAGTAAAAAT TTTTTTGCAT TTTCACAAAT
 2751 TATACTTTAT ATAAGGCATT CCACACCTAA GAACTAGTTT TGGGAAATGT
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 2851 TTTGGCTAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA
 2901 AAAAA

Fig. 1 (cont.)

REFVERB0 Q90970, 76_153
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 PTAS_HUMAN_72_146
 PPAR_HUMAN_100_174
 ROR1_HUMAN_71_146
 RRB2_HUMAN_79_154
 RRA1_HUMAN_86_161
 MTL1_Q64104_14_91
 HNF4_HUMAN_49_124
 RRXA_HUMAN_133_208
 RRXH_HUMAN_201_278
 COT1_HUMAN_84_159
 EBR2_HUMAN_101_176
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 PRGR_HUMAN_565_640
 GCR_HUMAN_419_494
 ANDR_HUMAN_557_632
 STER_FACT1_Q2052388_11_86
 NGFI_HUMAN_265_340
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 VDR_HUMAN_22_97
 VDR_BOVIN_19_94
 VDR_COTJA_42_117
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 VDRRg_dbd
 Q19333_120_195
 Q14994_9_84
 LXR_ALPHA_Q13133_96_171
 OR1_RAT_Q62755_76_151
 FXR_Q62735_122_197
 ECR_DROME_262_317
 PHA1_HUMAN_51_125
 PHA2_HUMAN_51_128

Fig. 2

Evolutionary Neighbour-Joining Tree



1 MEVRPKESWN HADFVHCEDT ESVP GKPSVN ADEEVGGPQI CRVCGDKATG
51 YHFNVMTC EG CKGFFRRAMK RNARLC PFRR KGACEITRKT RRQCQACRLR
101 KCLESGMKKE MIMSDEAVEE RRALIKRKKS ERTGTQPLGV QGLTEEQRMM
151 IRELMDAQMK TFDTTFSHF K NFR LPGVLSS GCPELPSLQA PSREEAAKWS
201 QVRKDLCSLK VSLQLRGEDG SVWNYKPPAD SGGKEIFSL PHMADMSTYM
251 FKGIISFAKV ISYFRDLPIE DQISLLKGAA FELCQLRFNT VFNAETGTWE
301 CGRLSYCLED TAGGFQQLL EPMLKFHYML KKLQLHEEEY VLMQAISLFS
351 PDRPGVLQHR VVDQLQEQA ITLKS YIECN RPQPAHRFLF LKIMAMLT EL
401 RSINAQHTQR LLRIQDIHPF ATPLMQELFG ITGS

Fig. 4

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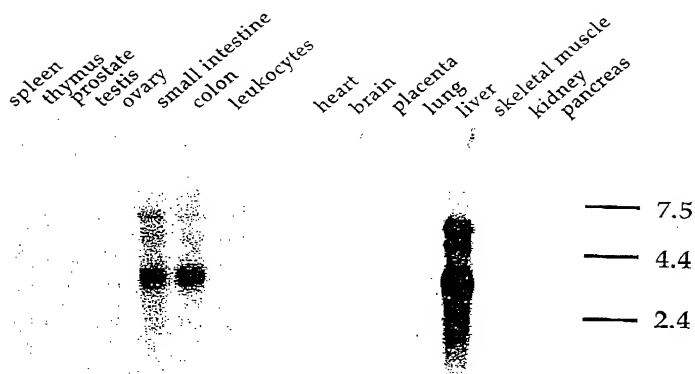


Fig. 5

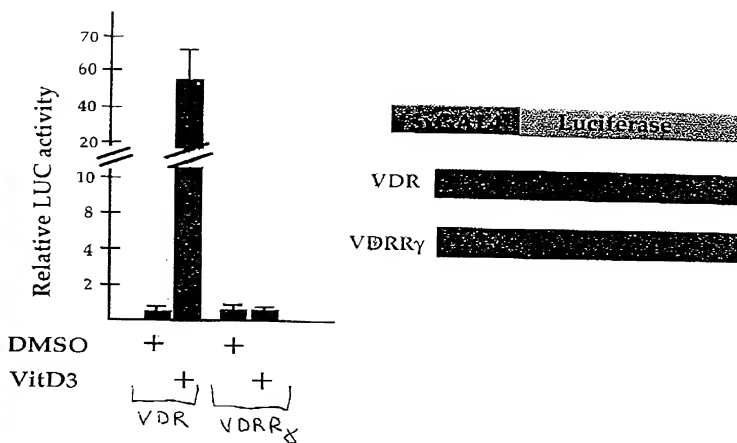


Fig. 6

TGAATTGCTGGGCTGCTGGGTTAGTGTGGCAGCCCCC 40
 TGAGGCCAAGGACAGCAGCATGACAGTCAACGAGACTCAC 80
 CACTTCAAGGAGGGGTCCCTCAGAGCACTTGCCATACCCC 120
 TGCCACAGTGTCTGGGCTGAGTTGGCTTCAAAACCATCCAAG 160
 AGGCCCCAGAGCAAAACCTGGAGGTGAGACCCAAAGAAAGC 200
 TGGAAACATGCTGACTTTTGTACACTGTGAGGACACAGAGT 240
 CTGTTCCCTGGAAAGCCCAGTGTCAACGCAGATGAGGAAGT 280
 CGGAGGTCCCCAAATCTGGCGTGTATGTGGGGACAAGGCC 320
 ACTGGCTATCACTTCAATGTCAATGACATGTGAAGGATGCA 360
 AGGGCTTTTTTCAGGAGGGCCATGAACGCCAACGCCCGGCT 400
 GAGGTGCCCTTTCCGGAAGGGCGGCTTGGAGATCAACCGG 440
 AAGACCCGGGCGACAGTGCAGGGCCCTGCCGCCCTGGCCAAGT 480
 GCTTGAGAGCGGGCATGAAGAAGGAGATGATCATGTCCGA 520
 CGAGGCCGTGGAGGAGAGGGGGCTTTGATCAAGCGGAAG 560
 AAAAGTGAACGGACAGGGACTCAGCCACTGGGAGTGCAGG 600
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 TGACCTGTAGGTACAGGACCATCAGAGAGGCCAAGGTTGGCC 1760
 TTTCCCTTTTAAAAGGCCCTGTGGTCTGGGGAGAAATCCCT 1800
 CAGATCCCCTAAAGTGTCAAGGTGTGGAAGGGACCAAGC 1840
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 ACGTTGTTCGGCTTCCTGAGTCTTTTTCATTGCTACCTCTA 1920
 ATAGTCTCTGCTCCCACTTCCCACTCGTTCCCTCTCTCTT 1960
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 GGTGCATGAGTATCTGTGGGAGTCTCTAGAGAGATGAGA 2040
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 CTAGTTTGTGGAAATGTAGCCCTGGGTTTAATGTCAAAATC 2720
 AAGGCCAAAAGCAATTAATAATGTACTTTTGGCTAAAAAA 2760
 AA 2800
 AA 2802

Fig. 7 (cont.)

MTVTRTHHFKEGSLRAPAIPLHSAAAELASNHPRGPEANL 40
 EVRPKESWNHADDFVHCEDTESVPGKPSVNADEEVGGPQIC 80
 RVC GDKATGYHFNVMTCEGCKGFFRRAMKRNRARLRCPFRK 120
 GACEITRKTRRQCQACRLRKCLESGMKKEMIMSDEAVEER 160
 RALIKRKKCERTGTQPLGVQGLTEEQRMIMRELMDAQMKT 200
 FDTTFSHFKNFRLPGVLSSGCELPESLQAPSREEAAKWSQ 240
 VRKDLCSLKVSLQLRGEDGSVWNYKPPADSGGKEIFSLLP 280
 HMA DMSTYMFKGIISFAKVISYFRDLPTEDQISLLKGA AF 320
 ELCQLRFNTVFN AETGTWECGRLSYCLEDTAGGFOQLLLE 360
 PMLKFHYMLKKLQLHEEYVLMQAI SLFSPDRPGVLQHRV 400
 VDQLQEQFAITLKS YIECNRPQPAHRFLFLKIMAMLT ELR 440
 SINAQHTQRLRLRIQDIHPFATPLMQELFGITGS. 474

Fig. 8

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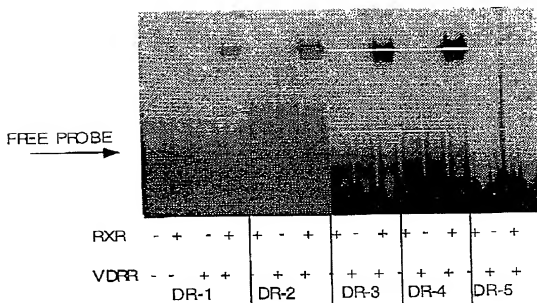


Fig. 9

12/15

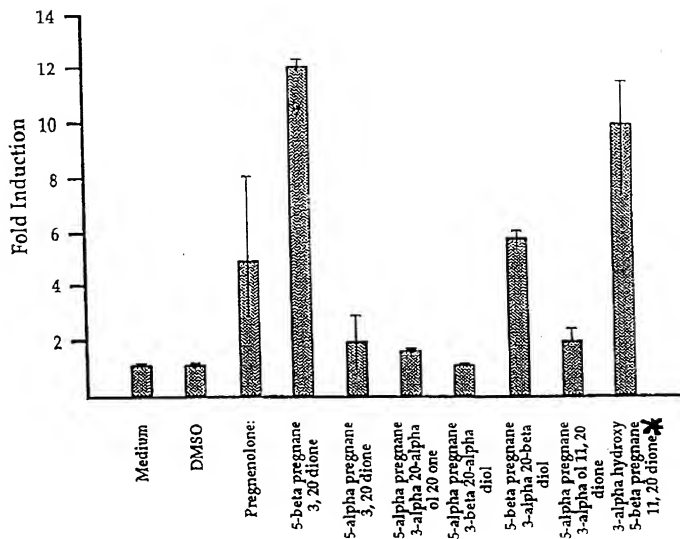


Fig. 10

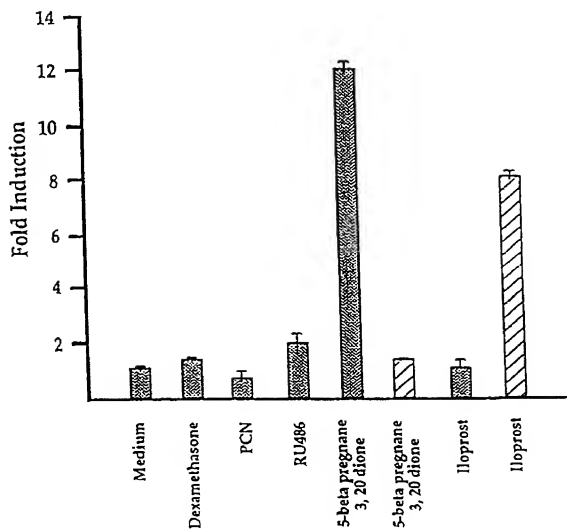


Fig. 11

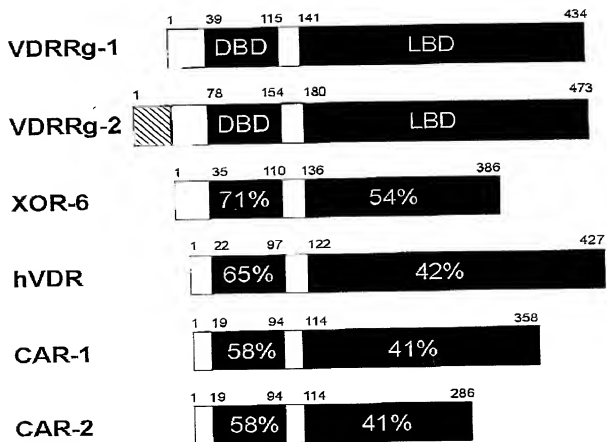


Fig. 12

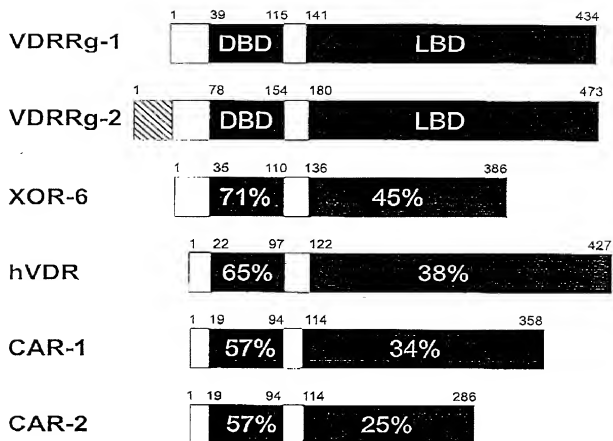


Fig. 13